FORM PTO-1390 (REV. 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

JC20 Rec'd PCT/PTO SEP 2 1 2001

ATTORNEY'S DOCKET NUMBER
MSK.P-038

US APPLICATION NO (If known, see 37 CFR 1 5
09/937192

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/09512	07 April 2000	09 April 1999

TITLE OF INVENTION
Methods and Compositions for Degradation and/or Inhibition of HER-Family Tyrosine Kinases

APPLICANT(S) FOR DO/EO/US
Sloan-Kettering Institute for Cancer Research

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☐ Other items or information:

EL556130416US

JC16 Rec'd PCT/PTO SEP 21 2001

				CALCULATIONS	PTO USE ONLY
21. ☒ The following fees are submitted: **BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):** Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO .... **$1000.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .... **$860.00** International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... **$710.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) .... **$690.00** International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**					
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 100.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	25 - 20 =	5	x **$18.00**	$ 90.00	
Independent claims	- 3 =		x **$80.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ 190.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$ 95.00	
SUBTOTAL =				$ 95.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$	
				Amount to be refunded:	$
				charged:	$

a. ☒ A check in the amount of $ 95.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. __________ in the amount of $ __________ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 15-0610 . A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

021121

PATENT TRADEMARK OFFICE

Marina T Larson

SIGNATURE

Marina T. Larson, Ph.D.

NAME

32,038

REGISTRATION NUMBER

09/937192
JC16 Rec'd PCT/PTO SEP 2 1 2001

MSK.P-038
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Rosen et al.
Serial No.: to be assigned
Filing Date: herewith
For : Methods and Compositions for Degradation and/or Inhibition of HER-Family Tyrosine Kinases

PRELIMINARY AMENDMENT

09937192.092101

Asst. Commissioner for Patents

Washington, D.C. 20231

Sir:

Preliminary to the calculation of the filing fee for the national phase application which is filed herewith, please make the following amendments:

In the specification:

On Page 1, after the Title, insert:

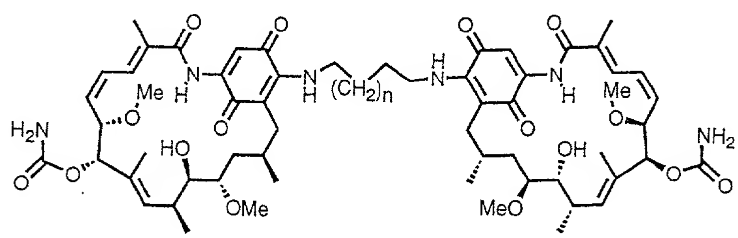
This application is a 371 of International Application No. PCT/US00/09512, filed April 7, 2000, which claims the benefit of US Provisional Application No. 60/128,593 filed April 9, 1999.

On Page 2, please amend the partial paragraph starting on line 21 to read a follows:

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Over-expression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is

associated with a poor prognosis. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

Page 3, please replace the structure at lines 19-24 with the following structure:



Dimers: n = 1- 9

Page 5, please replace the first partial paragraph and the first full paragraph with the following:

cause them to undergo homodimerization or heterodimerization with other members of the family. This activates the tyrosine kinase activity of the constituents of the dimer, causes their autophosphorylation and initiates transduction of the mitogenic signal. Although a direct interaction of hsp90 and HER-kinases has not been convincingly demonstrated, the fact that sensitivity of HER2 and other kinases to geldanamycin requires the catalytic domain of the kinase suggests that hsp90 is likely to interact with the catalytic domain of HER-kinases. As HER-kinase heterodimers are quite sensitive to GM, we speculated that each element of the heterodimer interacts with hsp90. Accordingly, it is believed that the dimers of the invention interact with both subunits of the HER-kinase heterodimers and thus more effectively and specifically target the active form of the HER-kinase. The mechanism of action appears to be

based on degradation of the HER-kinases, but may include or in some cases be derived entirely from an inhibition of activity of the HER-kinases.

Fig. 2 shows various compounds which have been synthesized and tested for activity and selectivity as promoters of tyrosine kinase degradation. The compounds tested included geldanamycin, geldanamycin homodimers with linkers of varying lengths, species with quinone or ring-opened geldanamycin linked to geldanamycin and geldanamycin coupled to a linker with no substituent at the other end. The linker in each case is bonded to carbon-17 of the geldanamycin moiety or moieties. The crystal structure of GM bound to hsp90 shows that carbon-17 is the only one not buried in the binding pocket. Stebbins, et al., *Cell* 89, 239-250 (1997).

<u>Page 7, please replace the paragraph starting on line 23 with the following:</u>

GMD-4c was also a potent inhibitor of the growth of breast cancer cells containing HER-kinases (Table 1) with an IC50 of 100 nM against MCF-7 compared to IC50 25 nM for GM and 650 nM for the one-ring opened dimer GMD-a. SKBR3 in which HER2 is highly overexpressed was also found to be very sensitive to GMD-4c. Most epithelial cancer cell lines express one or more members of the HER-kinase family. In order to assess whether the effects of GMD-4c on cells were specific, we utilized the 32D hematopoietic cell line. None of the members of the HER-kinase family are expressed in this murine IL3-dependent myeloid progenitor cell line. Wang, et al., *Proc Natl Acad Sci (USA)* 95, 6809-6814 (1998). GM is a potent inhibitor of 32D; GMD-4c does not appreciably affect its growth.

<u>On Page 8, please replace the first paragraph with the following:</u>

Based on these experimental results, we conclude that GMD-4c induces the selective degradation and/or inhibition of HER-family kinases and specifically inhibits the growth of HER-kinase containing tumor cell lines. This work supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the

presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

On Page 9 of the specification, please replace the partial paragraph starting on line 1 as follows:
100% dimethylsulfoxide (DMSO) and stored at -20°C prior to use in preparation of the geldanamycin derivatives of Fig. 2. The GM analogs were prepared according to the procedure depicted in Fig. 1, which is modified from the method of Schnur et al., *J. Med. Chem.* 38, 3813-3820 (1995). Briefly, the geldanamycin dimers (GMDs) were prepared by treatment of GM with 0.5 eq. of the appropriate diamine in DMSO. The ansa ring-opened GMDs (GMD-a and GMD-aa) were prepared by methanolysis (NaOMe/MeOH) of the GMD-4c. GM-quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

In the Figures:

Please replace figures 1 and 2 with the enclosed replacement sheets.

In the claims:

Please cancel claims 1, 2, 5 and 8 without prejudice and amend claims 3, 9, 12 and 13 to read as follows:

3. (amended) A chemical compound comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

6. (amended) The chemical compound of claim 4, wherein the linker has a length of 4 to 7 carbon atoms.

9. (amended) The chemical compound of claim 3, wherein the first and second hsp-binding moieties are geldanamycin.

12. (amended) A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

13. (amended) A method for treating cancer in a patient suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

Please add claims 15-29 as follows:

15. The method according to claim 13, wherein at least one of the hsp-binding moieties is geldanamycin.

16. The method according to claim 15, wherein the linker has a length of 4 to 7 carbon atoms.

17. The method according to claim 16, wherein the linker has a length of 4 carbon atoms.

18. The method according to claim 13, wherein the first and second binding moieties are geldanamycin.

19. The method according to claim 18, wherein the linker has a length of 4 to 7 carbon atoms.

20. The method according to claim 19, wherein the linker has a length of 4 carbon atoms.

21. The method according to claim 14, wherein at least one of the hsp-binding moieties is geldanamycin.

22. The method according to claim 21, wherein the linker has a length of 4 to 7 carbon atoms.

23. The method according to claim 22, wherein the linker has a length of 4 carbon atoms.

24. The method according to claim 12, wherein the first and second binding moieties are geldanamycin.

25. The method according to claim 24, wherein the linker has a length of 4 to 7 carbon atoms.

26. The method according to claim 25, wherein the linker has a length of 4 carbon atoms.

27. The method according to claim 12, wherein at least one of the hsp-binding moieties is geldanamycin.

28. The method according to claim 27, wherein the linker has a length of 4 to 7 carbon atoms.

29. The method according to claim 28, wherein the linker has a length of 4 carbon atoms.

REMARKS

This application is a national stage of PCT/US00/09512. In the International Preliminary Examination Report issued by the IPEA/US the Examiner indicated that claims 3-7 and 9-14 met the requirements of PCT Article 33(2)-(4). This amendment is filed to present only those claims.

Claims 1 and 2 have been cancelled, and claim 3 has been amended to independent form. Claim 5 has been cancelled as a duplicate of claim 9, and claim 6 has been amended to avoid dependency on a cancelled claim. Claim 8 has been cancelled and claim 9 has been amended to avoid dependency on a cancelled claim.

Claims 12 and 13 have been amended to include the recitation of the chemical composition from claim 3 and to eliminate multiple dependency. Claims 15-29 have been added to add the limitations of the dependent composition claims which had previously been included as a result of multiple dependency.

In addition, Applicants have made some minor amendments to the specification. These amendments add a reference to the parent PCT application, and correct several grammatical and clerical errors. No new matter is added by these amendments.

With respect to the replacement figure on Page 3, the replacement adds a double bond which was inadvertently omitted from one of the geldanamycin moieties, but which is included in the other. Since the specification clearly identifies the two parts of this molecule as being the same, and since the structure of geldanamycin was known, this is merely a clerical correction and is not new matter.

Applicants also enclose proposed replacement sheets for Figures 1 and 2, on which the proposed changes are highlighted. In Fig. 1, the changes include the addition of the missing double bond as noted above, changing the view so that the amide groups are more clearly depicted, and adding a subscript to the reagent $H_2N(CH_2)NH_2$ so that it correctly reads $H_2N(CH_2)_7NH_2$. The person skilled in the art would understand that a subscript had been omitted, and that the correct value is 7 since the product formed in the figure has a seven carbon linker. In Fig. 2, the changes alter the view so that the amide groups are more clearly depicted, and expand the carboxyl groups. It also indicates omitted orientation on one hydroxyl group, consistent with the orientation in the parent molecule. No new matter is added by these corrections.

In view of the indication that all of the claims now pending meet the requirements of PCT Article 33(2)-(4), Applicants submit that this application should be in form for allowance. Expedited handling in accordance with the provisions 37 CFR § 1.496(b) is respectfully requested.

Respectfully submitted,

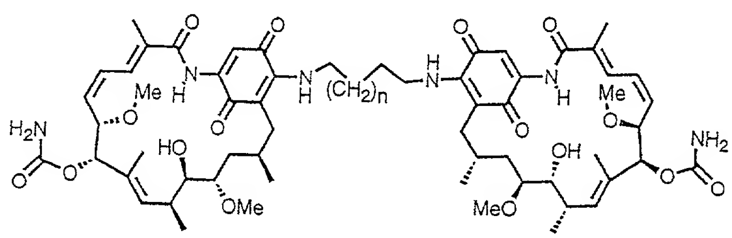
Marina T. Larson
Patent Office Reg. No. 32,038
Attorney for Applicants
(970) 468-6600 x 152

Marked Up Copy Showing Amendments

On Page 2 of the specification:

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Over-expression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is associated with a poor prognosis[2]. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

On Page 3 of the specification



Dimers: n = 1- 9

On Page 5 of the specification

cause them to undergo homodimerization or heterodimerization with other members of the family. This activates the tyrosine kinase activity of the constituents of the dimer, causes their autophosphorylation and initiates transduction of the mitogenic signal. Although a direct interaction of hsp90 and HER-kinases has not been convincingly demonstrated, the fact that sensitivity of HER2 and other kinases to geldanamycin requires the catalytic domain of the kinase suggests that hsp90 is likely to interact with the catalytic domain of HER-kinases. As HER-kinase heterodimers are quite sensitive to GM, we speculated that each element of the heterodimer interacts with hsp90. Accordingly, it is believed that the dimers of the invention interact with both subunits of the HER-kinase heterodimers and thus more effectively and specifically target the active form of the HER-kinase. The mechanism of action [is] appears to

be based [to at least] on degradation of the HER-kinases, but may include or in some cases be derived entirely from an inhibition of activity of the HER-kinases.

Fig. 2 shows various compounds which have been synthesized and tested for activity and selectivity as promoters of tyrosine kinase degradation. The compounds tested included geldanamycin, geldanamycin homodimers with linkers of varying lengths, species with quinone or ring[ed]-opened geldanamycin linked to geldanamycin and geldanamycin coupled to a linker with no substituent at the other end. The linker in each case is bonded to [the 17-carbon] carbon-17 of the geldanamycin moiety or moieties. The crystal structure of GM bound to hsp90 shows that [the 17-carbon] carbon-17 is the only one not buried in the binding pocket. Stebbins, et al., *Cell* 89, 239-250 (1997).

On Page 7 of the specification

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On Page 8 of the specification

Based on these experimental results, we conclude that GMD-4c induces the selective degradation and/or inhibition of HER-family kinases and [the] specifically inhibits the growth of HER-kinase containing tumor cell lines. This work[s] supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

On Page 9 of the specification

100% dimethylsulfoxide (DMSO) and stored at -20°C prior to use in preparation of the geldanamycin derivatives of Fig. 2. The GM analogs were prepared according to the procedure depicted in Fig. 1, which is modified from the method of Schnur et al., *J. Med. Chem.* 38, 3813-3820 (1995). Briefly, the geldanamycin dimers (GMDs) were prepared by treatment of GM with 0.5 eq. of the appropriate diamine in DMSO. The ansa ring-opened [ansaring-opened] GMDs (GMD-a and GMD-aa) were prepared by methanolysis (NaOMe/MeOH) of the GMD-4c. GM-

quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

In the Figures:

Please replace figures 1 and 2 with the enclosed replacement sheets.

In the claims:

3. (amended) A [The] chemical compound[according to claim 2] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

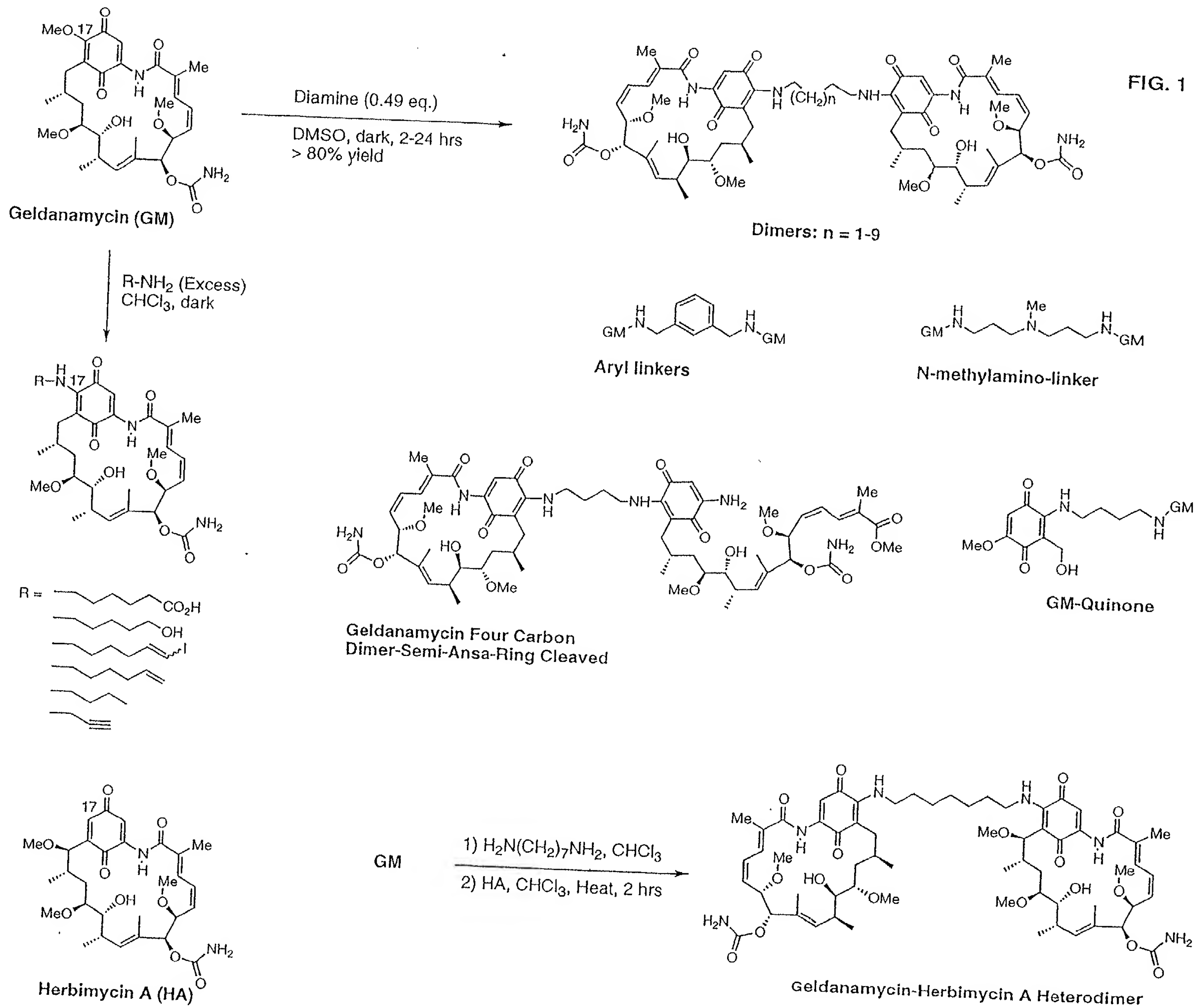
6. (amended) The chemical compound of claim [5] 4, wherein the linker has a length of 4 to 7 carbon atoms.

9. (amended) The chemical compound of claim [8] 3, wherein the first and second hsp-binding moieties are geldanamycin.

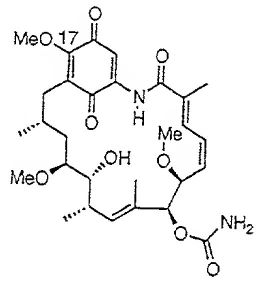
12. (amended) A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

13. (amended) A method for treating cancer in a patient[s] suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound [according to any of claims 1-11] comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

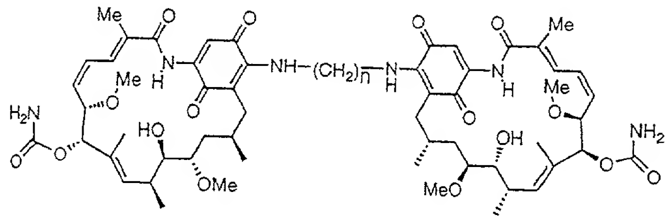
FIG. 1



Geldanamycin (GM)
Diamine (0.49 eq.)
DMSO, dark, 2-24 hrs
> 80% yield
Dimers: n = 1-9
R-NH2 (Excess)
CHCl3, dark
Aryl linkers
N-methylamino-linker
Geldanamycin Four Carbon
Dimer-Semi-Ansa-Ring Cleaved
GM-Quinone
Herbimycin A (HA)
GM
1) H2N(CH2)7NH2, CHCl3
2) HA, CHCl3, Heat, 2 hrs
Geldanamycin-Herbimycin A Heterodimer



Geldanamycin (GM,O)



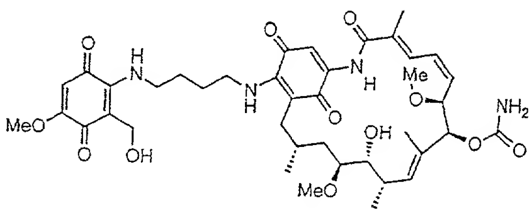
GM dimers (GMD, O——O)

n = 4: GMD-4C, O–4–O

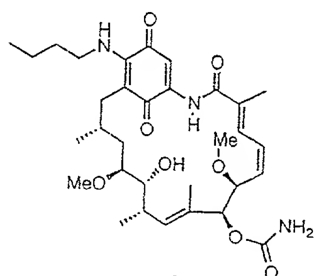
n = 7: GMD-7C, O–7–O

n = 9: GMD-9C, O–9–O

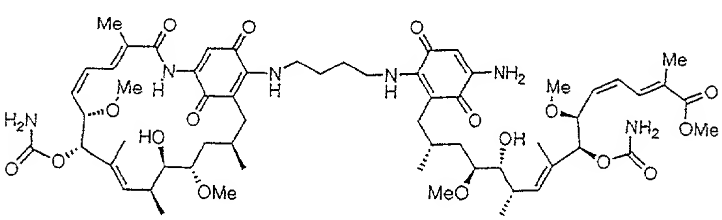
n = 12: GMD-12C, O–12–O



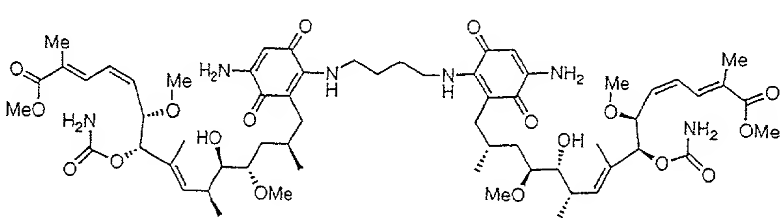
GM-Quinone, O–4–□



GM-linker, O–4–



GMD-a, O–4–≺



GMD-aa, ≻–4–≺

Fig. 2

METHODS AND COMPOSITIONS FOR DEGRADATION AND/ OR INHIBITION OF HER-FAMILY TYROSINE KINASES

TECHNICAL FIELD

This application relates to bifunctional molecules having two moieties which interact with the chaperone protein hsp90, including in particular homo- and heterodimers of ansamycin antibiotics. These bifunctional molecules act to promote degradation and/or inhibition of HER-family tyrosine kinases and are effective for treatment of cancers which overexpress Her-kinases.

BACKGROUND ART

Targeted delivery of therapeutic agents as a means for treating cancer has been proposed by many authors. Conceptually, the idea is to deliver a toxic substance selectively to the cancer cells, thus reducing the general toxicity to the patient. This is theoretically possible, since many cancer cell types have been found to have increased levels of hormone receptors and similar receptors. For example, breast cancer cells may have elevated levels of HER2 receptors or estrogen receptors which result in hormone-stimulated growth of cancer cells, while androgen receptors are required for growth of many prostate cancers and mutation of the androgen receptors frequently occurs in advanced prostate cancer.

Hormone receptors have been used in studies on the feasibility of using direct targeted chemotherapy agents to certain classes of cells. Thus, for example, Lam et al., *Cancer Treatment Reports* 71: 901-906 (1987) have reported on estrogen-nitrosourea conjugates as potential cytotoxic agents against human breast cancer, while Brix et al., *J. Cancer Res.* 116: 538-539 (1990) have reported on studies of the use of androgen-linked alkylating agents as antineoplastic agents. See also, Eisenbrand et al., *Acta Oncologica* 28: 203-211 (1989). Myers and Villemez, *Biochem. Biophys. Res. Commun.* 163: 161-164 (1989) have disclosed the possibility of utilizing luteinizing hormone coupled to a truncated diphtheria toxin.

Geldanamycin (GM) and herbimycin A (HA) are natural benzoquinoid ansamycin antibiotics. Uehara, et al., *Mol. Cell. Biol.* 6, 2198-2206 (1986). This class of drugs binds to a specific pocket in the chaperone protein hsp90. Stebbins, C.E. et al., *Cell* 89, 239-250 (1997); Prodromou, et al., *Nat Struct Biol* 4, 477-482 (1997). Occupancy of

this pocket by the drug leads to the degradation in the proteasome of a subset of proteins that require hsp90 for conformational maturation. Schneider, et al., *Proc. Natl. Acad. Sci. (USA)* 93, 14536-14541 (1996); Csermely, et al., *Pharmacol Ther* 79, 129-168 (1998); Scheibel, et al., *Biochem Pharmacol* 56, 675-682 (1998). These include the HER- and insulin-receptor family of transmembrane tyrosine protein kinases, Raf serine kinase and steroid receptors. Addition of GM to tumor cells leads to an Rb-dependent G1 growth arrest and apoptosis. HER-kinases are among the most sensitive targets of GM and tumor cell lines in which HER2 is overexpressed are inhibited by especially low concentrations of the drug. Miller, et al., *Cancer Res* 54, 2724-2730 (1994); Schnur, et al., *J. Med. Chem.* 38, 3806-3812 (1995); Hartmann, et al., *Int J Cancer* 70, 221-229 (1997). These findings imply that GM and related drugs may be useful in the treatment of a variety of tumors. An analog of GM, 17-allyaminoGM, will soon be studied in phase I clinical trials. However, the number of important signaling molecules that are affected by ansamycins suggests that they may have untoward toxicity. In addition, benzoquinoid antibiotics can induce the selective degradation *in vivo* of receptors, including estrogen, androgen and progesterone receptors. International Patent Publication No. WO98/51702 discloses the ability of compositions comprising an ansamycin antibiotic or other hsp90 binding moiety and a targeting moiety which specifically binds to a target protein to stimulate the selective degradation of such target proteins, and the use of such compositions as chemotherapy agents.

HER-family transmembrane receptor tyrosine kinases play an important role in transducing extracellular growth signals and when activated can be oncogenic. Tzahar, et al., *Biochim Biophys Acta* 1377, M25-37 (1998); Ross, et al., *Stem Cells* 16, 413-428 (1998). Overexpression of HER1 and HER2 occurs in a variety of human malignancies. Amplification of the HER2 gene is a common event in human breast and other carcinomas and, in breast cancer, is associated with a poor prognosis2. HER1 and HER2 are attractive targets for therapeutic development. Antibodies against each of these receptors have been shown to have antitumor effects in animal models. Fan, et al., *Curr Opin Oncol* 10, 67-73 (1998). Recently, an anti-HER2 antibody was shown to be effective in the treatment of breast cancers in which the HER2 protein is overexpressed. Ross et al., *supra*; Pegram, et al., *J Clin Oncol* 16, 2659-2671 (1998). However, therapeutic effects were seen in only a minority of patients and were usually short-lived. It is not known whether this is due to

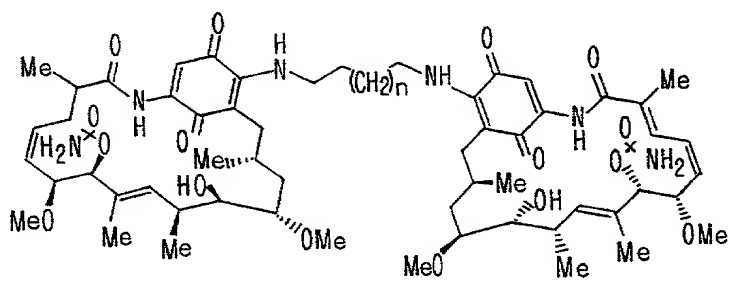
inefficient inhibition of the target by the antibody, but clearly other more effective methods for HER2 inhibition are needed.

Thus, notwithstanding efforts to date, there remains a substantial need for compositions which can be utilized to selectively target and degrade HER-family tyrosine kinases which can be used as chemotherapy agents in the treatment of HER-expressing cancers, including Her2 positive breast cancer. It is an object of the present invention to provide such compositions.

It is a further object of the present invention to provide a method for the treatment of Her-positive cancers.

DISCLOSURE OF THE INVENTION

We have now found that bifunctional molecules comprising two linked hsp-binding moieties which bind to hsp90 in the pocket to which ansamycin antibiotics bind are effective for inducing the degradation and/or inhibition of HER-family tyrosine kinases. For example, a composition having the structure



*Dimers: n = 4

provides selective degradation and/or inhibition of HER-family tyrosine kinases, without substantially affecting other kinases. Thus, the compositions of the invention can be used for treatment of HER-positive cancers with reduced toxicity, since these compounds potently kill cancer cells but affect fewer proteins than geldanamycin.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a synthetic scheme for the preparation of compounds in accordance with the invention;

Fig. 2 shows the structures of various compounds synthesized and tested for activity and specificity of action against HER-family tyrosine kinases; and

Fig. 3 shows the effect of geldanamycin and GMD-4c on 32D cells was evaluated after treatment with various concentrations.

MODES FOR CARRYING OUT THE INVENTION

The compounds of the invention are a new class of bifunctional molecules comprising two hsp-binding moieties that bind to hsp90 in the pocket to which ansamysin antibiotics bind, connected to each other by a linker. Preferred compounds are those in which at least one, and more preferably both of the hsp-binding moieties are ansamysin antibiotics such as geldanamycin or herbimycin.

The two hsp-binding moieties are joined by a linker. As discussed in more detail below, this linker may be of varying lengths. Altering the length of the linker results in different activity levels and specificity profiles. In general, the linker will be 1 to 9 carbon atoms in length, and may be a linear carbon chain or a substituted carbon chain, for example incorporating double or triple bonds, an aryl group or a secondary or tertiary amine. (See. Fig. 1)

Compounds in accordance with the invention can be synthesized in accordance with the scheme set forth in Fig. 1. In the case of a homodimer, reaction of an ansamycin antibiotic such as geldanamycin with a diamine of appropriate length and structure in DMSO results in the preparation of homodimers in substantial yield. For preparation of a heterodimer, the ansamycin antibiotic such as geldanamycin is first reacted with a primary monoamine, having a second, non-amine functional group. The resulting product is in turn covalently coupled via this functional group to the second type of hsp-binding moiety. An example of such a heterodimer which can be made by this method is a geldanamycin-herbimycin heterodimer.

The compounds of the invention are effective to induce degradation and/or inhibition of HER-family tyrosine kinases, but have a narrower spectrum of action and greater selectivity than, for example geldanamycin *per se*. While not intending to be bound by any particular mechanism of action, the activity and selectivity of the compounds of the invention may arise from the fact that extracellular growth factors that bind to HER-kinases

cause them to undergo homodimerization or heterodimerization with other members of the family. This activates the tyrosine kinase activity of the constituents of the dimer, causes their autophosphorylation and initiates transduction of the mitogenic signal. Although a direct interaction of hsp90 and HER-kinases has not been convincingly demonstrated, the fact that sensitivity of HER2 and other kinases to geldanamycin requires the catalytic domain of the kinase suggests that hsp90 is likely to interact with the catalytic domain of HER-kinases. As HER-kinase heterodimers are quite sensitive to GM, we speculated that each element of the heterodimer interacts with hsp90. Accordingly, it is believed that the dimers of the invention interact with both subunits of the HER-kinase heterodimers and thus more effectively and specifically target the active form of the HER-kinase. The mechanism of action is appears based to at least on degradation of the HER-kinases, but may include or in some cases be derived entirely from an inhibition of activity of the HER-kinases.

Fig. 2 shows various compounds which have been synthesized and tested for activity and selectivity as promoters of tyrosine kinase degradation. The compounds tested included geldanamycin, geldanamycin homodimers with linkers of varying lengths, species with quinone or ringed-opened geldanamycin linked togeldanamycin and geldanamycin coupled to a linker with no substituent at the other end. The linker in each case is bonded to the 17-carbon of the geldanamycin moiety or moieties. The crystal structure of GM bound to hsp90 shows that the 17-carbon is the only one not buried in the binding pocket. Stebbins, et al., *Cell* 89, 239-250 (1997).

These compounds shown in Fig. 2 were evaluated as described in the examples below, and assessed in terms of efficiency of induction of degradation of the HER2 and Raf protein kinases in MCF-7 cells. The results are summarized in Table 1, where GM moiety is designated as 'O'; carbon linker, as '-'; quinone, as '☐ '; the GM moiety with an opened ansaring, as '> ' or ' < '. As shown, the properties of the geldanamycin dimers vary as a function of linker chain length. GM itself causes the induction of HER2 degradation with an IC50 of 45 nM. Dimers with linkers of four to seven carbons retain activity against HER2 (IC50 60-70 nM). Dimers with longer linkers lose activity; the twelve carbon-linked compound has an IC50 of 750 nM.

Table 1
The effects of geldanamycin analogs on the steady-state levels of HER-2 and Raf-1 and the cell growth of MCF-7.

Drug[a]	IC50[a] (HER-2) (nM)	IC50[b] (Raf-1) (nM)	IC50[c] (growth inh.) (nM)
O	45	200	25
4 O——O	60	3200	100
4 O——<	500	3500	650
4 >——<	>5000	>5000	>2000
7 O——O	70	500	600
9 O——O	500	3800	700
12 O——O	750	3500	700
4 O——	80	350	600
4 O——□	55	350	350

Each of the geldanamycin dimers tested showed increased specificity for HER-2 over Raf-1, when compared to GM. The four carbon-linked dimer (GMD-4c) has the greatest selectivity of the compounds tested. GM causes Raf degradation with an IC50 of 200 nM. GMD-4c is much less active, with an IC50 of 3200 nM. Selectivity decreases with increasing chain length; the seven carbon linked-dimer (GMD-7c) retains activity against HER2 (IC50 70 nM) and is only slightly less active than GM against Raf (IC50 500nM). As linker carbons increase to more than eight, activity against both targets declines in parallel.

The properties of GMD-4c were examined in greater detail by immunoblotting. GM causes the degradation over time of HER-kinases, Raf-1, estrogen receptor and, more slowly, IGF-I receptor. The GMD-4c hybrid molecule reduces HER2

expression with the same kinetics both on Western blot and by immunohistochemical analysis. However, under these conditions GMD-4c does not affect Raf-1 or IGF-I expression. Estrogen receptor levels declined transiently but returned to baseline by 24 hours. A faster migrating HER2-immunoreactive band appeared after 12 hr, predominantly after treatment with GMD-4c. This form accumulates in intracellular vesicles and corresponds to immature HER2. Glycosylation studies revealed that this HER2 form is partially glycosylated and sensitive to endoglycosidase H.

Additional GM derivatives were synthesized to explore the mechanism of selectivity, and the results are summarized in Table 1. ButylaminoGM (GM-linker), a molecule in which the four-carbon linker is attached to one geldanamycin residue, and a four carbon-linked heterodimer of GM and a quinone (GM-quinone), are modestly weaker than GM against both HER2 and Raf-1; they are not selective. GMD-aa, a GMD-4c in which the ansa ring of both of the GM moieties is opened is inactive. GMD-a, a dimer in which the ring of only one of the GM moieties is open has much reduced activity against both targets (IC50 HER2 500 nM, IC50 Raf-1 3500 nM). These data suggest that the selectivity of GMD-4c depends on both GM moieties.

This apparent selectivity could be a property of a weaker or more rapidly metabolized drug which might appear to have selective activity against the most sensitive target (HER2). To address this question, GM and GMD-4c were added to cells at different concentrations and frequencies. Even when GMD-4c was added at high concentrations four times in twelve hours, it retained selectivity. Thus, the rate of metabolism does not appear to be the reason for the observed selectivity

GMD-4c was also a potent inhibitor of the growth of breast cancer cells containing HER-kinases (Table 1) with an IC50 of 100 nM against MCF-7 compared to IC50 25 nM for GM and 650 nM for the one-ring opened dimer GMD-a. SKBR3 in which HER2 is highly overexpressed was also found to be very sensitive to GMD-4c. Most epithelial cancer cell lines express one or more members of the HER-kinase family. In order to assess whether the effects of GMD-4c on cells were specific, we utilized the 32D hematopoietic cell line. None of the members of the HER-kinase family are expressed in this murine IL3-dependent myeloid progenitor cell line. Wang, et al., *Proc Natl Acad Sci (USA)* 95, 6809-6814 (1998). GM is a potent inhibitor of 32D; GMD-4c does not appreciable affect its growth.

Based on these experimental results, we conclude that GMD-4c induces the selective degradation and/or inhibition of HER-family kinases and the specifically inhibits the growth of HER-kinase containing tumor cell lines. This works supports the idea that selective ansamycins with a different, more restricted spectrum of targets than the parent molecules can be synthesized. In this case, the mechanism of selectivity is not yet known, but depends on the presence of both GM moieties and is a function of the length of the linker. GMD-4c may selectively interact with HER-kinase heterodimers, but it is also possible that it preferentially interacts with different hsp90 family members than GM.

HER-kinases are overexpressed in many human tumors, including many breast, ovarian, pancreatic and gastric cancers, and are likely to be important in maintaining the growth of some of them. Anti-HER2 antibodies have activity in the treatment of some breast cancers that overexpress the protein, but the responses are usually partial and of modest duration. The reasons the antibody is of limited utility are unknown, but may relate to incomplete inhibition of HER-kinase function. If so, drugs that destroy these kinases will be much more effective. For this reason, 17-allylamino-geldanamycin, a non-selective ansamycin, is about to be studied in phase 1 trial. GMD-4c is a compound that is effective in destroying HER-kinases, but is likely to be much less toxic than GM, since its effects on other key signaling proteins are attenuated. This represents a new strategy for abrogating growth receptor function in human tumors.

Thus, in accordance with the invention, patients, including human patients having tumor cells that overexpress HER-family tyrosine kinase are treated by administering to the patient a therapeutically effective dose or doses of a chemical compound comprising linked first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind. Preferred compounds are those in which at least one of the hsp-binding moieties is an ansamycin antibiotic such as geldanamycin. GMD-4c is most preferred. The specific dosage amount utilized will reflect a balancing of efficacy and toxicity, and can be determined only through clinical trials which have not been conducted. The establishment of trial protocols and the determination of the appropriate dosages and treatment frequency is a matter within the skill in the art.

EXAMPLE 1

Geldanamycin was kindly provided by Drs. David Newman and Edward Sausville (Drug Synthesis and Chemistry Branch, National Cancer Institute), dissolved in

100% dimethylsulfoxide (DMSO) and stored at -20°C prior to use in preparation of the geldanamycin derivatives of Fig. 2. The GM analogs were prepared according to the procedure depicted in Fig. 1, which is modified from the method of Schnur et al., *J. Med. Chem.* 38, 3813-3820 (1995). Briefly, the geldanamycin dimers (GMDs) were prepared by treatment of GM with 0.5 eq. of the appropriate diamine in DMSO. The ansaring-opened GMDs (GMD-a and GMD-aa) were prepared by methanolysis (NaOMe/MeOH) of the GMD-4c. GM-quinone was synthesized by first treating GM with excess 1,4-diamobutane, then addition of 2-methoxy-1-hydroxymethylquinone.

For the specific synthesis of the GMD-4c, the following procedure was followed. To a mixture of 10 mg (0.0178 mmol) of geldanamycin in 0.2 mL of DMSO was added 0.94 mL of 1,4-diaminobutane (0.5 eq.). After stirring for 4 hrs in the dark at room temperature, the reaction was judged complete by TLC. The mixture was concentrated and purified via flash chromatography on silica gel (5% MeOH/CH_2Cl_2) to afford 8.5 mg of dimer product (85%).

For the specific synthesis of a geldanamycin-herbimycin dimer, the following procedure was followed. To a mixture of GDM (6.1 mg, 0.01 mmol) in 1.5 mL of $CHCl_3$ was added 11.6 mg (10 eq.) of 1,6-diaminohexane in a 2 mL vial. The reaction was stirred at room temperature for one hour in the dark. The chloroform was then washed with water (3 x 2 mL) and concentrated. To this mixture was added 8.6 mg (0.015 mmol) of herbimycin A and 0.5 mL of CH_2Cl_2. The mixture was heated to 40°C for 24 hours in a sealed vial. The mixture was purified directly via flash chromatography on silica gel (5-7% MeOH/ CH_2Cl_2) to afford 1.2 mg of C-19 and 3.4 mg C-17 linked (herbimycin numbering) heterodimers (45%).

EXAMPLE 2

The human breast cancer cell lines MCF-7 and SKBR-3 were obtained from ATCC (Rockville, MD), and maintained in DME/F12 (1:1) supplemented with 10% heat-inactivated fetal bovine serum (Gemini Bioproducts), 2 mM glutamine, and 50 units/ml each of penicillin and streptomycin, in a humidified 5% CO_2/air atmosphere at 37°C.

Polyclonal antibodies against HER2 (c-18), Raf-1 (c-12), and PI3 kinase (p85) (Z-8) were purchased from Santa Cruz Biotechnology, Inc. A monoclonal antibody against estrogen receptor (clone H-151) was obtained from StressGen Biotechnology Corp. A polyclonal antibody against the β-subunit of IGF-I receptor was kindly provided by Dr. L-H. Wang (Mt. Sinai Medical Center, New York).

Immunoprecipitation and immunoblotting was used to evaluate activity and selectivity of geldanamycin derivatives. MCF-7 cells were exposed to 1μM geldanamycin or derivative for periods of 3 to 24 hours. After exposure, cells were washed twice with ice-cold phosphate-buffered saline (PBS), collected by scraping and transferred into microcentrifuge tubes. For immunoblotting (HER2, Raf-1, ER), cells were lysed with SDS lysis buffer (50 mM Tris-HCl, pH 7.5, 2% SDS, 10% glycerol, and 1 mM DTT), boiled for 10 min, and sonicated briefly; for immunoprecipitation (IGF-IR), cells were lysed with NP40 lysis buffer (50 mM Tris-HCl, pH 7.5, 1% Nonidet P-40, 150 mM NaCl, 1 mM Na_3VO_4, 40 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 µg/ml each of aprotinin, leupeptin and soybean trypsin inhibitor) for 20 min at 4°C. Cell lysates were cleared by centrifuging at 14,000 x g for 15 min at 4°C in a microcentrifuge; the supernatants were collected as the experimental samples. Protein concentration in each sample was determined using the BCA kit (Pierce) according to the manufacture's instructions. For detecting IGF-IR, samples were immunoprecipitated with anti-IGF-IR antibody. Immunocomplexes were collected on protein A-Sepharose beads (Pharmacia), washed three times with the lysis buffer. Samples were subjected to SDS-PAGE, electrotransferred to nitrocellulose membranes, detected using the ECL kit (Amersham) according to the manufacturer's protocol, and quantitated using the Gel Doc 1000 (Bio-Rad). IC50 for protein degradation is designated as the amount of each drug needed to degrade 50% of the protein (HER2 or Raf-1) compared to the control in MCF-7 cells after 24-hr treatment.

For cell growth experiments, cells were plated in 6-well tissue-culture plates (Corning) at 20,000 cells/well. Two days after plating, cells were treated with different concentrations of drugs or the vehicle DMSO (0.1%). MCF-7 cells were treated for four days; medium with the appropriate drug or vehicle was changed every 2 days; cells were trypsinized, collected and counted on a Coulter counter; IC50 for cell growth is designated as the amount of each drug needed to inhibit MCF-7 growth by 50% compared to the control vehicle.

Numerical results for IC50 for HER-2, Raf-1 and cell growth are summarized in Table 1, with each number representing the average of three different experiments. GM causes the degradation over time of HER-kinases, Raf-1, estrogen receptor and, more slowly, IGF-I receptor. The GMD-4c hybrid molecule reduces HER2 expression with the same kinetics both on Western blot and by immunohistochemical

analysis. However, under these conditions GMD-4c does not affect Raf-1 or IGF-I expression. Estrogen receptor levels declined transiently but returned to baseline by 24 hours.

EXAMPLE 3

To evaluate a possible explanation for the observed selectivity of GMD-4c for degradation of HER-kinase, cells were treated with either geldanamycin or GMD-4c at concentrations of 0.25, 0.5 and 1 μM in varying treatment patterns as follows: single treatment at time 0, treatments at 0 and 3 hours, treatments at 0, and 6 hours, treatments at 0 and 12 hours and treatments at 0, 3, 6 and 12 hours. No significant differences were observed in the amount of HER-2 and Raf-1 as a function of the treatment pattern.

EXAMPLE 4

Murine hematopoietic cell line 32D, which lacks HER-kinases, was kindly provided by Dr Yosef Yarden (The Weizmann Institute of Science, Israel), and maintained in RPMI-1640 supplemented with 10% heat-inactivated FBS, 2 nm interleukin 3 (R&D systems), 2 mM glutamine, and 50 units/ml each of penicillin and streptomycin.

The effect of geldanamycin and GMD-4c on 32D cells was evaluated after treatment with various concentrations of compound by counting the number of cells every day for three consecutive days using a hematocytometer. The results are summarized in Fig. 3. As shown, geldanamycin was very toxic to these cells, but GMD-4c was much less so, thereby confirming the selectivity of action of GMD-4c against cells expressing HER-family tyrosine kinases.

EXAMPLE 5

MCF-7 cells were grown and treated on fibronectin-coated coverslips placed in multiwell plates. Cells were fixed for 20 min at -20°C in 100% methanol, rehydrated in PBS for 10 min at room temperature and blocked for 30 min at 37°C in a blocking solution consisting of 2% bovine serum albumin (BSA), 10% normal goat serum and 0.05% Tween-20 in PBS. HER2 and Raf-1were immunodetected with antibodies from Santa Cruz (SC284 and SC133, respectively); IGF-IR, an antibody from Calbiochem Oncogene Science (Ab1). Slides were incubated with a 11000 dilution of the primary antibody in blocking buffer for 1 hr at room temperature, followed by an incubation with Alexa 546-coupled goat anti-rabbit

IgG secondary antibody (A-11010, Molecular Probes) at a 150 dilution in blocking buffer, or fluoresceine isothiocyanate-coupled goat anti-mouse IgG (F276, Molecular Probes) at a 1100 dilution. Slides were washed three times with 1 ml of 0.5% BSA in 0.05% Tween-20 in PBS in between and after incubations with primary and secondary antibodies. DNA was stained with bisbenzimide, which was included in the secondary antibody solution (3 μg/ml final concentration). Coverslips were mounted on glass slides using Vectashield (Vector), to prevent quenching of fluorescence. Immunofluorescence was detected with a Zeiss epifluorescence microscope, at 40X and 100X, using appropriate filters for detection of rhodamine and bisbenzimide, and via confocal microscopy.

Substantial staining was observed for all three antibodies in the DMSO control, while reduced staining was observed for all three antibodies in cells treated with geldanamycin. For cells treated with GMD-4c, significant reduction in fluorescence levels was only observed for the anti-HER2 antibodies.

We claim:

1. A chemical compound comprising first and second hsp-binding moieties which bind to the pocket of hsp90 with which ansamycin antibiotics bind, said binding moieties being connected to one another by a linker.

2. The chemical compound according to claim 1, wherein the first hsp-binding moiety is an ansamycin antibiotic.

3. The chemical compound according to claim 2, wherein the first and second hsp-binding moieties are each an ansamycin antibiotic.

4. The chemical compound of claim 3, wherein at least one of the hsp-binding moieties is geldanamycin.

5. The chemical compound of claim 4, wherein the first and second hsp-binding moieties are geldanamycin.

6. The chemical compound of claim 5, wherein the linker has a length of 4 to 7 carbons atoms.

7. The chemical compound of claim 6, wherein the linker has a length of 4 carbon atoms.

8. The chemical compound of claim 1, wherein at least one of the hsp-binding moieties is geldanamycin.

9. The chemical compound of claim 8, wherein the first and second hsp-binding moieties are geldanamycin.

10. The chemical compound of claim 9, wherein the linker has a length of 4 to 7 carbons atoms.

11. The chemical compound of claim 10, wherein the linker has a length of 4 carbon atoms.

12. A method for destruction of cells expressing a HER-family tyrosine kinase, comprising administering to the cells a chemical compound according to any of claims 1-11.

13. A method for treating cancer in a patients suffering from cancer, comprising administering to the patient a therapeutic composition comprising a chemical compound according to any of claims 1-11.

14. The method of claim 13, wherein the cancer is an HER-positive cancer.

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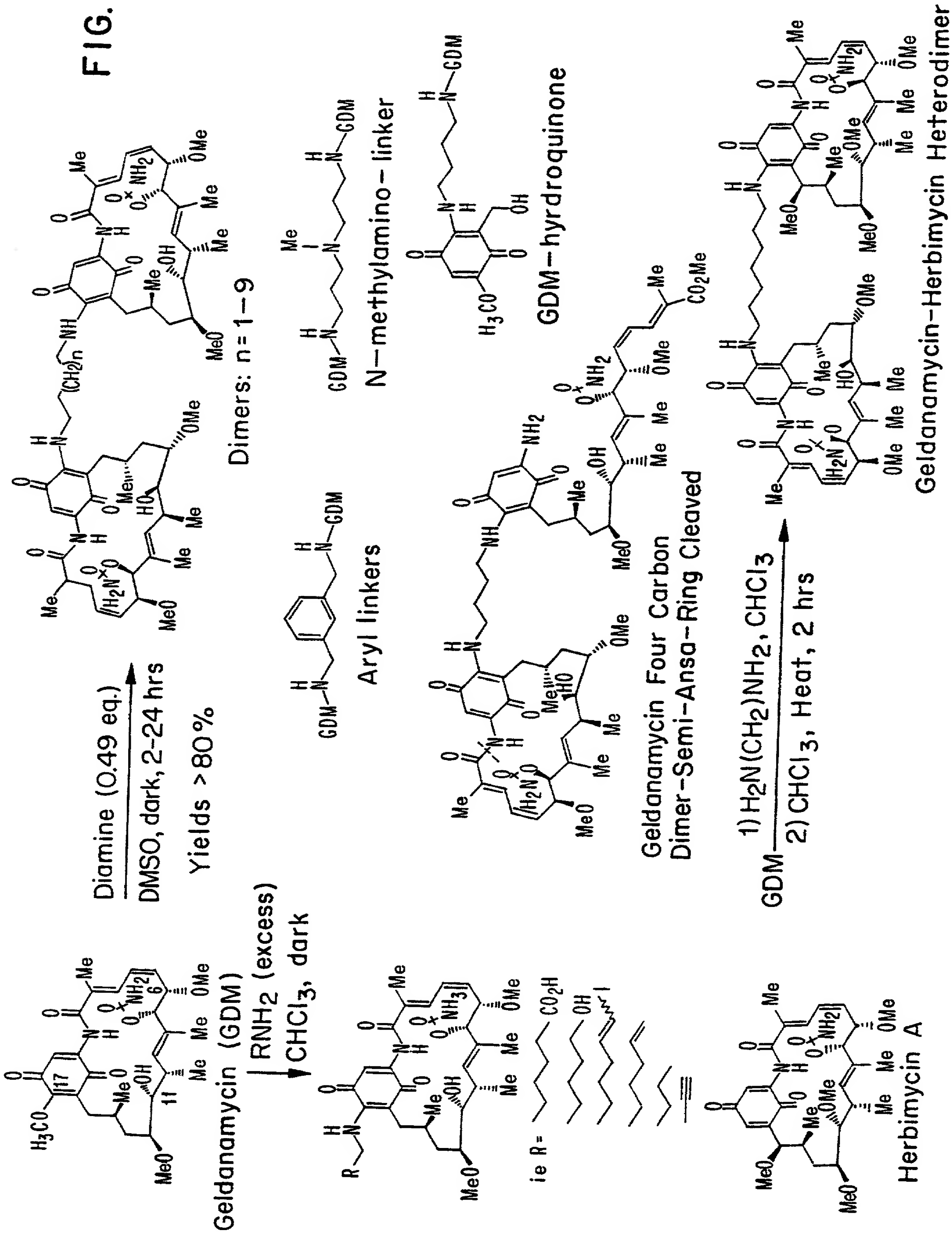
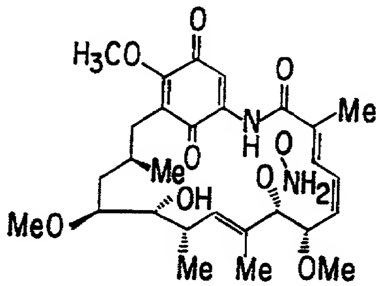
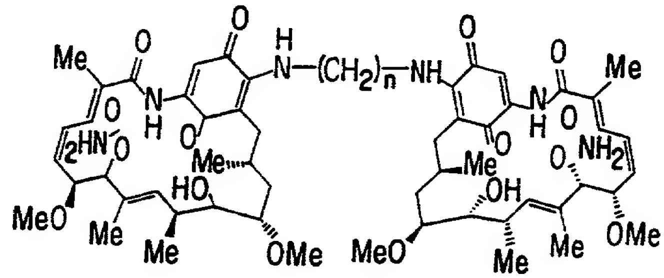
FIG. 1

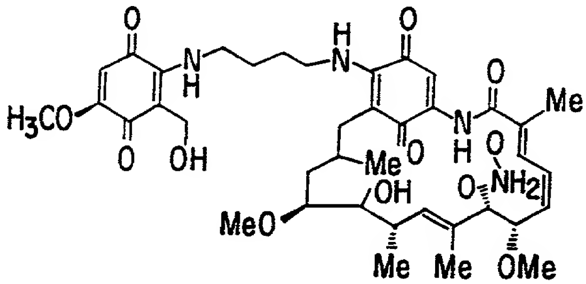

Geldanamycin (GDM)
RNH2 (excess)
CHCl3, dark
ie R=
Herbimycin A
Diamine (0.49 eq.)
DMSO, dark, 2-24 hrs
Yields >80%
Dimers: n = 1—9
Aryl linkers
N—methylamino—linker
GDM—hyrdroquinone
Geldanamycin Four Carbon
Dimer-Semi-Ansa-Ring Cleaved
1) H2N(CH2)NH2, CHCl3
2) CHCl3, Heat, 2 hrs
Geldanamycin-Herbimycin Heterodimer



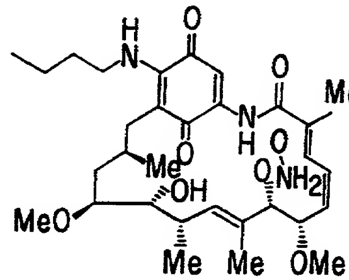
Geldanamycin (GM, O)



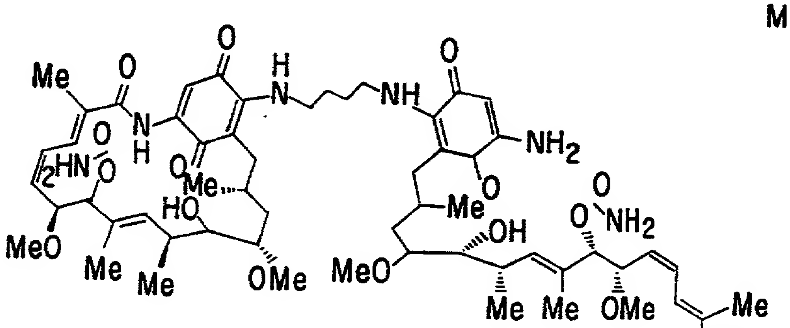
GM dimers (GMD, O—O)
(n=4: GMD−4c, O$\overset{4}{—}$O;
n=7: GMD−7c, O$\overset{7}{—}$O;
n=9: GMD−9c, O$\overset{9}{—}$O
n=12: GMD−12c, O$\overset{12}{—}$O



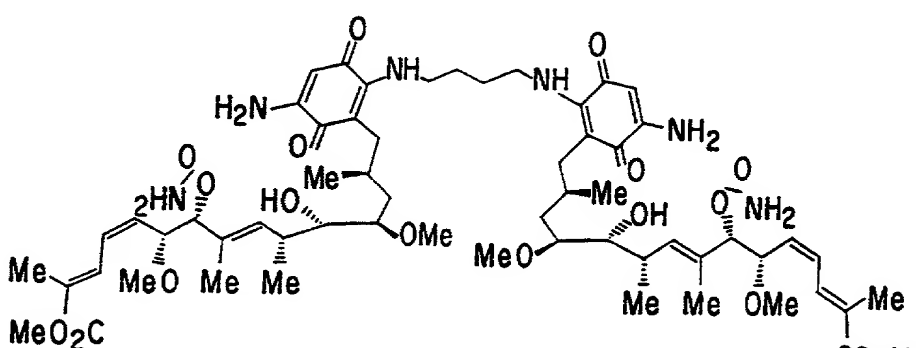
GM−quinone (O$\overset{4}{—}$□)



GM−linker (O$\overset{4}{—}$)



GMD−a (O$\overset{4}{—}$<)



GMD−aa (>$\overset{4}{—}$<)

FIG 2

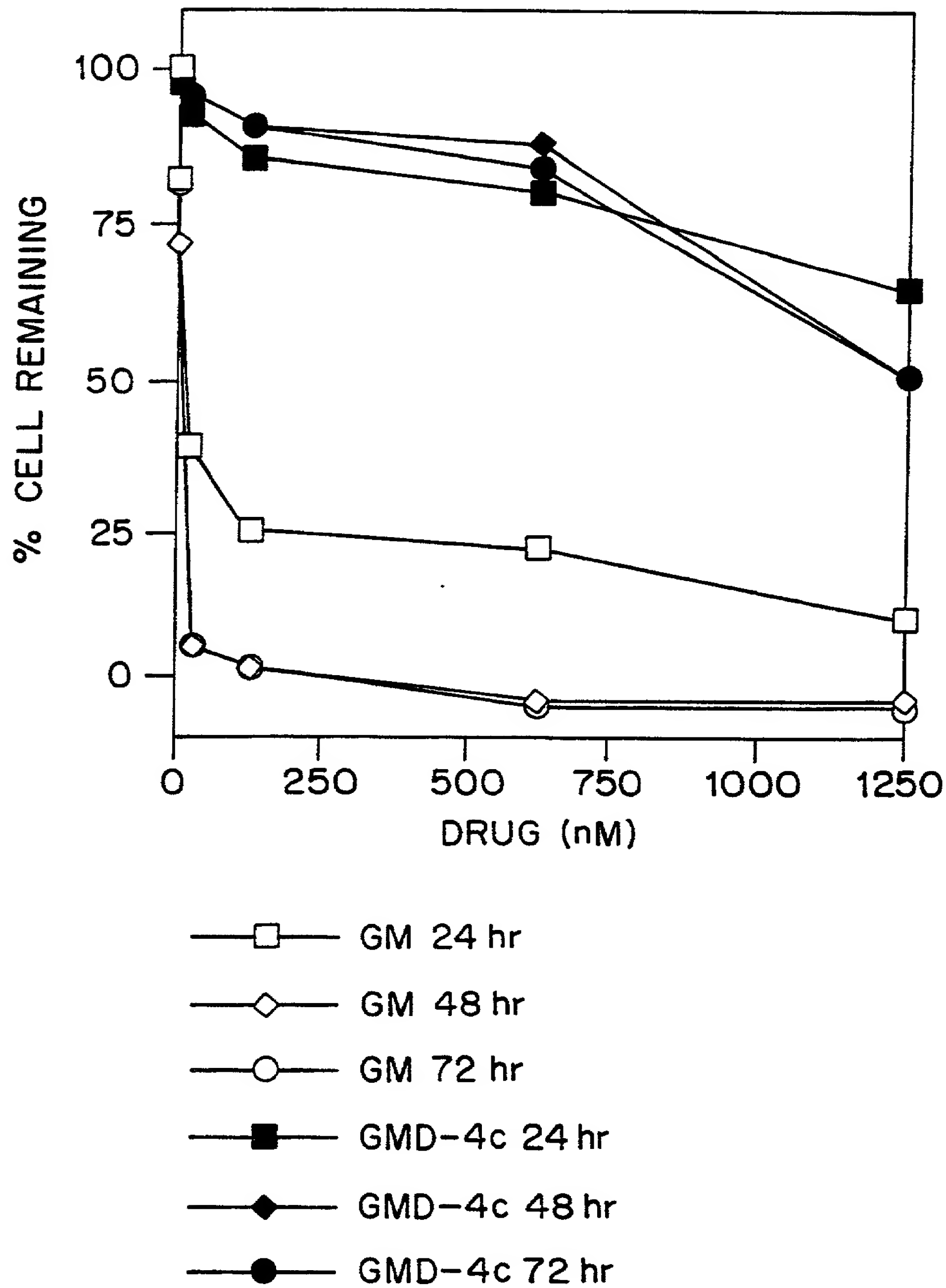

100
75
50
25
0
% CELL REMAINING
0
250
500
750
1000
1250
DRUG (nM)
GM 24 hr
GM 48 hr
GM 72 hr
GMD-4c 24 hr
GMD-4c 48 hr
GMD-4c 72 hr

FIG. 3

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My citizenship, residence and post office address are as listed below next to my name.

I believe I am the original, first and [ ] sole/[x] joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: **Fusion Receptors Specific for Prostate-Specific Membrane Antigen and Uses Thereof** the specification of which

(a) [ ] is attached hereto.

(b) [ ] was filed on________________ as Application Serial No. ____________________ and was amended on ___________.

(c) [X] was described and claimed in International Application No. PCT/US00/09512 filed on April 7, 2000.

Acknowledgment of Duty of Disclosure

I hereby state that I have reviewed and understood the content of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

35 U.S.C. § 120

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)
(Application Serial No.)	(Filing Date)	(Status)(patented,pending,abandoned)	(Patent No. if applicable)

Power of Attorney

3

I hereby appoint Carl Oppedahl, PTO Reg. NO. 32,746, Marina T. Larson, PTO Reg. No. 32,038, and D'Arcy Straub, Reg. No. 47,113, of the firm of OPPEDAHL & LARSON LLP, having office at P.O. Box 5068, Dillon, CO 80435-5068 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:	DIRECT TELEPHONE CALLS TO:
021121 PATENT TRADEMARK OFFICE	OPPEDAHL & LARSON LLP (970)468-6600

Claim for Priority

I hereby claim foreign priority benefits under 35 U.S.C. § 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign applications for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

EARLIEST FOREIGN APPLICATION(S), FILED WITHIN TWELVE MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION					
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	PRIORITY CLAIMED	CERTIFIED COPY ATTACHED
				YES[ ] NO[ ]	YES[ ] NO[ ]

FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day/month/year)	DATE OF ISSUE (day/month/year)	

Provisional Application

I hereby claim the benefit under 35 U.S.C § 119(e) of any United States provisional application(s) listed below.

60/128,593 — April 9, 1999

(application number) — (filing date)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR	LAST NAME Rosen	FIRST NAME Neal	MIDDLE NAME
RESIDENCE & CITIZENSHIP	CITY OF RESIDENCE Englewood	STATE OR COUNTRY OF RESIDENCE NJ	COUNTRY OF CITIZENSHIP USA
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DATE		SIGNATURE	

[X] Signature for additional joint inventor attached. Number of Pages 2

[ ] Signature by Administrator(trix) or legal representative for deceased or incapacitated inventor. Number of Pages ___.

[ ] Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 CFR § 1.47. Number of Pages __.

NAME OF SECOND INVENTOR	LAST NAME Kuduk	FIRST NAME Scott	MIDDLE NAME D.
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NAME OF THIRD INVENTOR	LAST NAME Danishefsky	FIRST NAME Samuel	MIDDLE NAME J.
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NAME OF FOURTH INVENTOR	LAST NAME Zheng	FIRST NAME Furzhong	MIDDLE NAME F.
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DATE 8/3/01		SIGNATURE	

NAME OF FIFTH INVENTOR	LAST NAME Sepp-Lorenzino	FIRST NAME Laura	MIDDLE NAME
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DATE 8/8/2001		SIGNATURE	

OUATHEK

NAME OF SIXTH INVENTOR	LAST NAME Ouerfelli	FIRST NAME Ouatek	MIDDLE NAME
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